Supplementary Information





Figure S1. Description of rats used in this study and quality of the genome assembly. (A) Overview of the HXB/BXH recombinant inbred rat family describing the origins and breeding history of the HXB/BXH rats. (B) BUSCO completeness of the genome assembly used to build the pangenome for genomics data quality control. Bar charts show proportions classified as complete (C, blues), complete single-copy (S, light blue), complete duplicated (D, dark blue), fragmented (F, yellow), and missing (M, red). (C) Nx plot showing assembly contiguity for each of the 31 rat strains.



Figure S2. Validation in the SHR/Olalpcv sample of the small variants called from the genome graph using vg. Small variants were validated over the joint-calling (JC) call set (gold standard) in the SHR/Olalpcv sample according to the scheme in **(A). (B)** Genome-wide accuracy of vg calls is ~100% in the easy regions for Single Nucleotide Polymorphisms (SNPs), ~90% in the easy regions and ~80% in the easy regions for Indels and hard regions of the genome. Exceptions are seen for chromosomes 16 and Y, which are enriched for complex variation **(C)**. Validation through Sanger sequencing was restricted to easy regions **(D)**, to SNPs **(E)** supported by original reads and in challenging, repetitive regions **(F)**.



Figure S3. Validation in the SHR/Olalpcv sample of the Structural Variants (SVs) called from the genome graph using vg. (A) Overlap of the call sets obtained by the three assembly-based methods (pav, svim, hall), and the graph-based method (vg) for chromosome 12 using the data from all rats. **(B)** Same as (A) for SHR/Olalpcv only. **(C)** Scheme of the validation for SVs



Figure S4. Integrated Genomic View of the validated Structural Variations (SVs). For each SV gray bars are validated reads mapped against the mRatBN7.2/rn7.fa reference genome, red bars define the boundaries of the SV.